CHEMICAL AND MICROBIOLOGICAL INVESTIGATION OF FEED MATERIALS, AND UTILIZATION OF OIL SEED CAKES FOR FOOD DEVELOPMENT

THESIS OF THE PHD DISSERTATION

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1. INTRODUCTION

The chemical and microbiological content of feeding stuffs make them be qualified. In the 1990s, the bovine spongiform encephalopathy (BSE) disease leading deterioration of nervous system in animals and in human was considered the most significant food safety problem. A direct correlation between feeding of inadequately heat treated feed materials from animal origin and the mad cow disease or the Creutzfeldt-Jacob disease (CJD) (FÜZI, 1999) led to reconsider the protection of humans, so chemical and microbiological contaminants in feeding stuffs of animals and in human foods got equal emphasis. Therefore, the "farm to table" concept has been adopted in the European Union food law, which states that the food cannot cause either directly or indirectly any damage to health (EUROPEAN PARLIAMENT AND THE COUNCIL, 2002). Due to the BSE scandal, the use of animal protein in feed with some exception became banned in the EU, which is still in force. Consequently, there was growing demand for feeding plant proteins, which could be realized through the by-products of oilseed processing and the oil seed meal like sunflower seed meal. At the same time, the development of functional foods has been launched with the aim of finding new methods to counteract the increasing number of patients suffering from of civilization related diseases (KAUR and DAS, 2011). A food can be regarded as functional if it is scientifically demonstrated to affect beneficially one of more target functions in the body, beyond adequate nutritional effects (BLADES, 2000). The curative effects of some oil seeds it is known even in the folk medicine (WESTCOTT and MUIR, 2003), and not surprisingly even in Hungary, the production and marketing of plant oils like those derived from pumpkin seed, linseed or walnut – citing their physiological effects – has boosted. Nowadays, there is increasing number of small-and-medium size enterprises (SMEs) that are processing of oil seeds for oil pressing at variable technical level (BÁNÁTI, 2003). The pressing residue (oil seed cake – OSC) is not further processed, and lately is used only for animal feeding. However, the pressing residue through its oil content and other compounds like lipid soluble vitamins and dietary fibres can improve the nutritional and biological value of innovative compound feeds. If the degree of chemical and microbiological pollutants conform the applicable legal limits, the OSCs could be used as a solid, flour component of functional foods. The requirements of food or feed use are consistent in some cases e.g. pathogen-free, and in many other cases they are different e.g. deterioration of microbiological origin, mycotoxin contamination etc.. In the field of human nutrition the scientific interests toward the use of OSC are ever-growing.

Comprehensive investigation for their food application can highlight that these materials can be appropriate as food components, and can be applied for designing functional foods.

2. OBJECTIVES

The objectives of my doctoral work were to evaluate the safety of feed materials and the products derived thereof and to make a comprehensive study of some feed materials, selected oil seed cakes to establish their application in the bakery industry.

To achieve this, the following subtasks were performed:

To evaluate the safety of plant origin feed materials and the products derived thereof:

1. Determination of mould count and mould genus
2. Quantification of selective mycotoxins (aflatoxin B1, ochratoxin-A, zearalenone, T-2)
3. Quantification of mesophilic microbial contamination of selective oil seed cakes covering a short term storage model experiment.

To study the potential of oil seed cakes as bakery ingredient:

1. Evaluate the nutritional value through determination the macro-composition of OSCs,
2. Determination of technological properties of OSC enriched wheat dough,
3. Baking trials and sensory evaluation of OSC enriched wheat breads,
4. Comparative study of OSC enriched wheat breads with traditional, marketed breads through objective measurements of colour and texture (hardness) of bread crumb.

3. MATERIALS AND METHODS

3.1. Measurements on the subjects of feed safety

Samples (Studied materials): grains and products derived thereof: Barley, What, Spelt, Maize Groats, Triticale, Avena, Wheat bran, feed wheat flour, Cottonseed, Soya bean, Soya bean meal, Sunflower seed meal, Rape seed meal, Walnut kernel cake, Linseed cake, Sunflower seed cake with hull.

Methods: *the mycotoxins* (Aflatoxin B1, Ochratoxin-A, Zearalenone, T-2 toxin) were determined by ELISA method, *the determination of mould count* were performed using traditional culturing methods laid down in the Quality Assurance Manual of Animal Health and Food Control Station of Szabolcs-Szatmár-Bereg County. The measurements were carried out at the institution here mentioned.

Microbiological measurements of oil seed cakes in storage experiment: The OSCs were stored at 4 °C, 14 °C and 25 °C for 4 months, in folded, opaque plastic bags, of which the air was displaced manually. Traditional culturing methods (EN standards) were applied to quantify the mesophilic microbiota depending on oxygen demand and spore-forming property (viz. the total mesophilic aerobic count, mesophilic aerobic spore-forming bacteria, yeast-mould count, total mesophilic anaerobic count, mesophilic anaerobic spore-forming bacteria, sulphite-reducing *Clostridia*, coliform count, *E.coli*). These measurements were performed at the Agricultural and Molecular Research and Service Institute of College of Nyíregyháza.

3.2. To study the potential of oil seed cakes as bakery ingredient

Samples (Studied materials): Grinded by-products of oil seed processing, obtained by cold pressing of walnut (*Juglans regia* L.), or yellow and brown linseed (*Linum usitatissimum* L.), or naked pumpkinseed (*Cucurbita pepo var. styriaca* L.), or sunflower seed (*Helianthus annuus* L.) were introduced to chemical measurements and were applied for wheat bread enrichment. The production of oil seeds and the oil processing were performed at Hungarian companies, including in particular Közép-Tiszai Mg. ZRt. (Kunhegyes), Solio Kft. (Szekszárd), and Tarpai Manufaktúra Kft. (Tarpa). To the comparative evaluation, control white bread and breads bought from the market like the half-brown bread as light-type bread, and brown-type breads: the rye-, and the multi-grain (whole-grain) bread were chosen. The control white bread was made from wheat flour (BL-80).

Methods

<u>Chemical measurements</u>: Moisture was determined according to MSZ 6496-93 standard; Protein was calculated as nitrogen content*6.25, to which nitrogen content was determined by Elementar Rapid N cube analyzer according to MSZ 16634-1 standard. Total dietary fiber and soluble fiber were determined according to the method of Megazyme Total Dietary Fibre Assay Procedure Kit (Noack Group).Total fat content was determined according to MSZ EN ISO 734-1:2000 standard; the fat composition was determined with Agilent Technologies 7890A gas chromatograph (FID) involving sample preparation according to MSZ 19928-89 standard. Ash was determined according to MSZ 6369/3-1987 standard. Mineral elements were determined after microwave digestion of samples with Perkin Elmer Optima DV 3000 ICP-OES instrument. Water activity, as a 5-minute constant value, was measured by Novasina labMaster-a_w Basic instrument. The measurements were performed as part of the project EA_KUTF-napraforgó05, at the Agricultural and Molecular Research and Service Institute of College of Nyíregyháza.

<u>Technological properties of OSC enriched dough</u> were determined with valorigraph, according to MSZ 6369/6-1988 standard in the laboratory of ABOMILL zRt. in Nyíregyháza.

<u>Baking trials of OSC enriched breads</u> and the control white bread were made according to plant practice. The determination of width/high ratio were performed according to MÉ 2-81, the sensory evaluation of baked breads involved description, 20-point-scoring, 10-judge ranking. These small-

scale experiments were performed at the bakery of Westsik Vilmos Technical and Secondary School of Food Industry, in Nyíregyháza. 5 and 10 % wheat flour were substituted with any studied OSC. Crumb hardness were measured with Brookfield LFRA Texture Analyser according to AACC 74-10.02 method, for the colorimetric measurements of bread crumb Colorlite sph 860 spectrophotometer was applied (instrument set: illuminant D65, 10 degree standard observer, data were given in CIE Lab and LCh colour system). The texture and colorimetric measurements were performed at the Agricultural and Molecular Research and Service Institute.

4. RESULTS

Chemical and microbiological characterization results of feed materials cereals, oilseeds and their by-products:

1. The average and the median value of mould contamination of cereal grains exceeded the limit of feed application. Of the studied samples, 50 % (N=10) in 2002, and 31% (N=13) in 2003 were non-compliant to safe feeding. The average mould contamination of oil seed meals was low, two order of magnitude, below the 1000 cfu/g threshold.
2. 43,5% of feed material samples was contaminated with mycotoxin below the detection limit of ELISA test applied. The mycotoxin level has not exceeded the limits in any incremental sample.

3. Presence of Salmonella was unlikely in the studied oil seed cakes (OSCs). Walnut cake (WnC), sunflower seed cake with hull (SC_{wh}), and in brown linseed cake (LC_b) were free from pathogen sulphite-reducing *Clostridia*, because these agents were not detected in them, and potentially were not present in dehulled sunflower seed cake (SC), yellow linseed cake (LC_y) and in hull-less pumpkin seed cake (PuC) through indication tests.

4. On the score of the storage experiment, the microbiological contamination of the studied OSCs was influenced by the product meaning the type of OSC ($P \leq 0.000$), the microbe group ($P \leq 0.000$), the repetition (batch) ($P \leq 0.001$).

   Ascending order of the average microbial contamination [log10 cfu/g]:

   1) *by the product*: WnC (2.73), SC_{wh} (3.27), LC_b (3.53);
   2) *by the microbe group*: aerobic sporogenic bacteria (2.39), fungal count (2.51), total aerobic microbe count (3.00), anaerobic sporogenic bacteria (3.75), and total anaerobic microbe count (4.23). These contamination values are in compliance with the Hungarian Health Ministry Regulation No 4/1998, from where the requirements of processed cereal products including bran were here applied as benchmark, due to the lack of official categorization of OSC as food.

5. Regarding the variation of microbial contamination during 4-month storage period, it was observed that the temperature (4°C, 14°C, 25°C) had no effect (P=0.191), while storage time seemed to be more influential (P=0.003).

6. The water activity of OSCs (WnC, LC_b, SC_{wh}) were below the critical value (0.7) for microbial propagation. These studied OSC samples had a 4-month shelf-life in folded, plastic packaging at 25°C.

intervals of water activity by OS, measured between 4-25°C during 4 month storage		
WnC	LC_b	SC_{wh}
0.50-0.54	0.59-0.60	0.53-0.57

7. The OSCs are rich in macronutrients:

sample name	protein	TDF	SF	fat	ash
Pumpkin seed cake (hull-less seeds)	50.4±0.5	23.2±0.5	16.2±0.2	8.2±0.3	7.2±0.3
Sunflower seed cake (dehulled)	48.5±0.4	24.9±0.6	20.7±0.2	9.1±0.5	7.0±0.1
Sunflower seed cake (with hull)	31.9±0.6	42.0±0.5	n.m.	23.6±0.6	6.4±0.2
Linseed cake (yellow)	34.5±0.2	32.4±0.3	17.6±0.3	14.3±0.4	5.4±0.2
Linseed cake (brown)	29.9±0.2	48.0±0.3	n.m.	18.9±0.4	7.1±0.2
Walnut cake	39.4±0.5	36.6±0.5	n.m.	15.5±0.2	4.9±0.8

data are given in g/100g; n.m.: not measured. TDF: total dietary fiber; SF: soluble fiber

8. Comparative evaluation of crumb colour between OSC enriched and light- or brown-type breads:
   1) Significant different were found (P<0.000) between LC_y or SC enriched breads and white bread.
   2) Addition of LC_y increased the yellowness, and result the same light-yellowish crumb at 5% addition, as the half-brown bread had. The colour parameters of these breads did not differed significantly, regarding L* values as lightness (P>0.441), b* values as yellowness (P>0.055), ΔL* values as colour difference (P>0.059), and the C values as vividness (P>0.061).
   3) Significant difference (P≤0.001) were found between the hue (h value) of WnC enriched bread and the brown type (rye- and multigrain) breads, probably due to the malt as an ingredient applied in the marketed breads.

9. Comparative evaluation of crumb texture between OSC enriched and light- or brown-type breads:

1) The same hardness were statistically confirmed:
   - in case of white and the 10% LC_y breads comparing the light-type breads (P=1.000). These were the softest ones;
   - in case of rye- and the WnC enriched breads, comparing the brown-type breads (P=0.113). In the rank of softness, these were the third softest ones (among the 6 groups got from statistical analysis);
   - in case of the studied addition ratios of WnC enriched breads (P=0.474);
   - in those cases when 5% of any studied OSCs were applied for enrichment comparing all the OSC enriched breads.
2) Increasing addition of PuC or SC resulted harder crumb (P=0.006).
3) The 10% SC enriched bread and the multi-grain bread were the hardest ones, with significant difference (P=0.000) comparing them.
4) The half-brown bread and the 10% SC bread had the same hardness (P=0.264).

4.1.THESIS

The following novel scientific results were obtained regarding the studies of food application of the selected OSCs:

1. WnC enrichment increased significantly the dough development time, making the dough hardly kneadable. A 5% and 10% addition of WnC increased the quality group of the composite wheat flour by 1 and 2 category units, comparing to the control wheat flour.
2. LC_y enrichment increased the dough development time. The industrial quality number of LC_y enriched flour did not changed comparing with the control wheat flour.
3. PuC enrichment did not influence the dough development time. substantially; however the dough stability were reduced. A 5 or 10% addition of PuC decreased the quality group of the composite flour by 1 category unit as compared to the control wheat flour.
4. SC enrichment reduced the dough development time and the dough stability. A 5% and 10% addition of SC decreased the quality of the composite flour by 1 and 2 category units, comparing with the control wheat flour.
5. Novel breads having a typical colouring and aroma can be produced using the studied OSCs for fortification. WnC fortified breads were brown; a green colour was obtained for PuC, yellowish for LC, and greyish-white or off-white for SC complementation.
6. The 10% WnC enriched bread was ranked in the first place (P=0.01), the control white got the last rank (P=0.01). The 10% LC enriched bread was ranked in the second place (P=0.05).

7. The 5% LC, WnC, PuC fortified breads had the same acceptability ($P=0.01$). The 5% SC enriched bread was the least plausible bread ($P=0.05$).
8. Through addition 5% and 10% of LC_y, breads having the same colour ($P>0.059$) but much softer ($P<0.000$) crumb than of the half-brown one can be produced.

5. CONCLUSIONS AND SUGGESTIONS

THE FEED SAFETY RESULTS SUGGEST that by the OSCs does not contain pathogenic bacteria like *Salmonella and* sulphite-reducing *Clostridia*.

The non-compliances laid bare in connection with microbial deterioration of feeds suggest that in the studied period the requirements of good manufacturing practice (GMP) for feed producers were fulfilled at variable levels, probably due to that the application of GMP was not obligatory at that time, furthermore the understanding, the management approach, and the obsolete technologies could be potential reasons.

Regarding the chemical contaminants, the risks of mycotoxins (Aflatoxin B1, Ochratoxin-A, Zearalenone, T-2 toxin) were also low. As a solution for lowering the mycotoxin contamination, good agricultural practice (GAP) and GMP can be applied, whose content are public and public-interest.

The scientific statement that the problem of undesirable chemical and microbiological substances in grains can be solved with prevention is accepted by the author of the present document. The risk of contamination can be lowered applying compliant growing, and prevention techniques and GMP before mentioned. These quality parameters have to be well-known by the factory staff and the management too, moreover their commitment toward quality is need to be adopted, developed and maintained.

Monitoring the chemical, biological and microbiological contaminants is necessary to be continued which contribute to achieve wider database for further risk assessment of the scientific committees in the field of food safety. This relevant data mass can be achieved by collaborations of business sector and research institutes, through publications, and we have possibilities to participate in opened European projects for data collecting.

THE RESULTS OF OSCS STUDIES SUGGEST that the OSCs have great potential not only as feed, but as food ingredient that is suggested by the followings:

1. ***OSCs have balanced macro-composition***. By their lipid- and protein content, OSCs have a higher energy content comparing with the cereal grains e.g. wheat, barley, rye, corn. Thanks to

the higher nutritional and biological value, the daily intake of a OSC enriched food can be lowered, because the dietary fibres provide the fed state earlier, and the protein- and lipid provide excess energy.

2. ***OSC enriched foods can have beneficial physiological effects***. It has been shown for liposoluble bioactive constituents like PUFA, vitamins and phytoestregenes that are also found in OSC enriched foods that could play a role in prevention of certain chronic diseases. The higher dietary fibre intake of OSC origin can contribute to lower the transition time and to increase the probability of prevention of colon cancer. OSCs provide increased mineral element content of foodstuff.
3. ***OSCs are alternative feed materials.*** By their high protein content, they can be alternative plant protein sources beside the allowed animal proteins, depending on the feeding purpose and efficacy results like maintaining, improving the health of breeding or laying hens flocks.
4. ***OSCs can be safe materials***, because the microbiological contamination can be kept at low level applying carefully the GMP system including the requirements of the processing and storage hygiene. The results of the laboratory storage experiment indicate that the analyzed OSCs can be safe and microbiologically stable materials for 4-month in folded, plastic packaging between 4°C and 25°C like at house-keeping volume and conditions. The type of developing food must be considered, because the spore-forming bacteria cause latent contamination making the high moisture content food products more risky. There is possible hazard of chemical contamination mostly by mycotoxins or heavy metals etc., not studied here, therefore monitoring of these substances are necessary.

THE OSCS COULD BE USED IN THE FOOD INDUSTRY FOR:

1. **Food fortification (enrichment)**: with the regards of biological content (lipid, dietary fiber, protein, minerals) OSCs can be applied presumably in the foods recommended daily in less quantities;
2. **Substitution of certain food texture modifiers;**
3. **Substitution of certain artificial food colouring agents or natural colorants;**
4. **Substitution of hydrogenated fat:** in high fat content cookies or cakes, the fat-composition of product directly can be modified toward vegetable oils introducing OSC into composite flour;
5. **Improvement of product yield:** dough with better technological properties can be achieved by composite flours consisting of WnC and poor quality (cheap) wheat flour;
6. **Isolation of pure constituents of natural origin:** e.g. protein isolate etc.
7. **Competitive bakery goods can be probably produced using OSC/wheat flour blends.** Beside the traditionally consumed bread like half-brown and brown breads, the OSC enriched

bread can enlarge the assortment. Beside labelling the OSC which provides added value and other basic and obligatory parameters, the widening of consumers' knowledge, the formation of consumers' approach can be realized at simple and economic way.

The above mentioned ideas should be pursued in order to promote food and feed research contributing to the development of research stuff and products in the field of OSC utilization. Furthermore, the development of OSC based functional foods is a novel research area in Hungary that could lead to higher productivity and economical indicators specific to the food industry, however, their physiological effects should be carefully assessed in comprehensive human-based trials.

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